Mathematical model and experimental design for human IgG diffusion

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Abstract: **A model of human IgG diffusion is presented. With the aid of computer based simulation, it will help members of the consortium of the EU funded project "Tissue engineered nerve repair devices: development of European medical implantable devices and research training focus" to predict the incorporation/release diffusion dynamics of soluted, including therapeutic antibody, in engineered tissues. The experimental design which will be used to identify the model parameters is also presented with the first experimental results.**

I Introduction

The overall aim of the EU funded project "Tissue engineered nerve repair devices: development of European medical implantable devices and research training focus" (acronym NEURAL TISSUE ENG) is to develop novel engineering bioartificial nerve grafts with which optimise and improve surgical peripheral nerve repair. This work will be assisted by the use of computer modelling to model the incorporation/release diffusion dynamics of soluted, including therapeutic antibodies.

Description of fibronectin and hyaluronan polymers
Nerve guides based both on fibronectin (Fn) and hyaluronan (HA) derivatives will be used within the project.
Unmodified HA exists only in the form of an aqueous gel and has short residence time in the cellular environment. Therefore, chemical derivatives of HA which retain the biocompatibility of the parent molecule while allowing it to be processed into defined physical forms, will be used.
HA derivative HYAFF-11 has been selected to be used as the first material to be tested for the bioartificial nerve prototype. HYAFF-11 is the benzyl ester of Hyaluronic Acid, i.e. with carboxyl groups of HA esterified with benzyl alcohol. By esterification of HA with alcohols of different chian lenghts, modified biopolymers can be produced, showing different physical-chemical properties from those of HA itself, but retaining the important biological activities of the parent molecule.
Purified HA was obtained directly from rooster comb, and used as starting material for the production of HYAFF. The molecular weight of the samples varies from 150.000 to 200.000 Daltons. The esters are prepared by treating a quaternary ammonium salt of HA with an esterifying agent in a suitable solvent at various controlled temperatures. The reasons for the selection of HYAFF-11 for the devices engineering step are first of all its well documented biocompatibility and biodegradability and secondly because its particular physical-chemical properties make it processable in various three dimensional forms.
Moreover, the esterification percentage of the carboxylic groups of HYAFF-11 can be controlled by the specific synthesis procedures. The higher the percentage of esterification of HA, the lower the solubility in water and the longer the degradation time in vivo. Among the HYAFF-11, therefore, the totally esterified derivative (HYAFF-11p100) and the 80% esterified derivative (HYAFF-11p80) for the devices engineering step were initially selected.
Observing the micro/macro-structure of the nerve guide device, three main elements form the final device:

- a Fn base tube
- HA-based glinding layers (in and outer)
- Central guiding fiber elements along which neural tissues should pass

II. Experiment planning for the diffusion model

The diffusion model is focused on the diffusion of human antibodies anti-TGFβ through bioengineered membranes. In order to characterise the uptake and release of anti-TGFβ1 to and from the Fn and HA materials used in the devices, the first experiments will be performed using IgG antibodies.
A literature survey of the mathematical models for macromolecules diffusion shows that great effort has been made for the study of the rate of diffusion in the local environment of macromolecules from polymers.
Polymers that release macromolecules, such as drugs for drug therapy, antibodies or antigens for immunoprotection, are useful for preventing and treating human diseases. The objective of these studies [1,2] was the determination of the diffusion coefficients in fluids (phosphate-buffered water, human mucus). The techniques used were fluorescence imaging of concentration profiles and fluorescence photobleaching recovery.

Materials and Methods:
Non-immunogenic neutralising human antibodies anti-TGFβ1 suitable for clinical use as locally derived therapies will be used in the project. The antibody is made commercially using recombinant technology by the Cambridge Antibodies Tecnology.
For the diffusion modelling study, however, standardised commercial IgG antibody preparation were used, since anti-TGFβ1 itself is structurally no different from IgG.
The experiments were done with IgG fluorescently labelled with two kind of detection in parallel in order to

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Abstract

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identify the best detection method according to reagents and instrumentation costs.
The tracing and diffusion quantitation of the IgG fluorescently labelled was performed by spectrofluorometry and in ELISA by using a commercial colorimetric kit named QuantiKin detection system.

Experiment planning:
A chamber suitable for the Fn tube through which the antibodies will diffuse was planned and constructed for the experiment (Fig.1).
The chamber contains cell culture medium supplemented with 5% foetal calf serum or, alternatively, physiological saline solution.
All the experiments were performed under sterilised conditions.
Samples (volumes ranging from 100 µl to 1 ml) were taken at different times from several sampling sites until the equilibrium was reached.

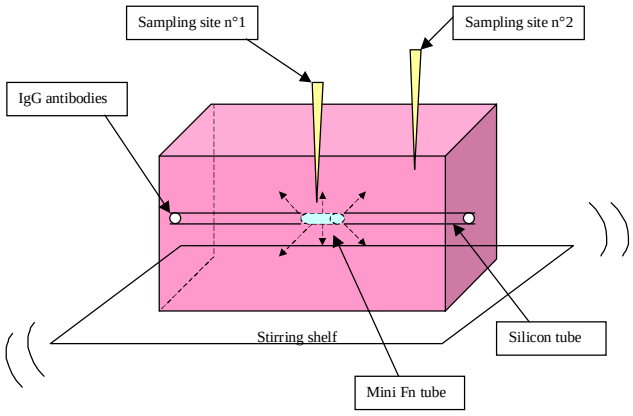

Fig. 1

Two experiment types were performed in parallel: experiments A with spectrofluorimetry detection and experiments B with colorimetric ELISA detection.

Spectrofuorimetry detection:
The spectrofluorimeter (Perkin Elmer) was gently supplied by Prof. Schettini of Advanced Biotechnology Centre, Genova, Italy. This instrument is directly driven by Windows NT specific software.

Colorimetric ELISA detection (Quantikin):
The quantitative kinetics software Quanti Kin has been developed for diagnostic colorimetric assays. The general characteristics are: applications for microplate based assays using unmodified kit protocols, connection with several microplate readers and user friendly interface because of the completely automatic procedure of the colorimetric detection.
Microplates with wells coated with mouse anti-human antibodies were used (Fig.2).

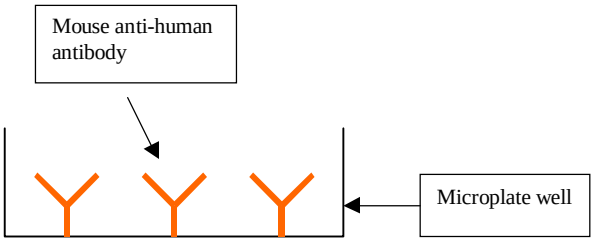

Fig. 2

Samples taken at different times from the diffusion chamber were added to microplate wells. After incubation for 15-20 min. at 4 °C, wells were washed with distilled water (Fig.3).

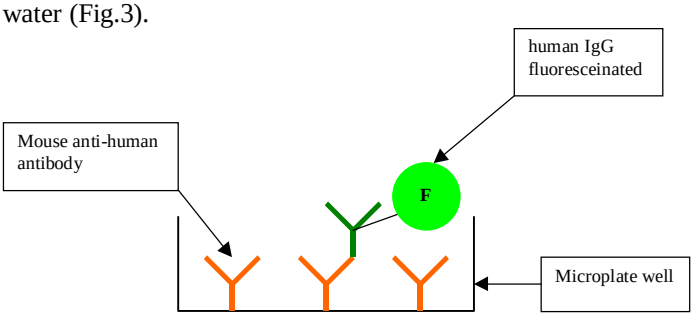

Fig. 3

Capture and detection phase is common to all specific applications of the Quanti Kin:

1) Capture on solid phase: the complex IgG-fluoresceina - mouse anti-human antibody will be captured by a monospecific purified anti-fluorescein antibody HRP-conjugated (Fig.4).

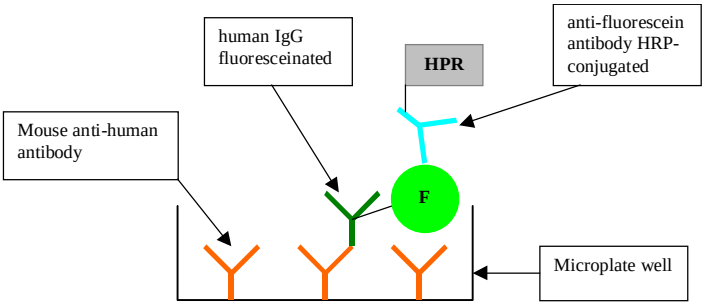

Fig. 4

2) Detection were performed by adding the substrate OPD (o-phenylenediamine dihydrochloride): HPR allows the colour development proportionally to the quantity of the IgG-fluorescein coniugated present (Fig.5).

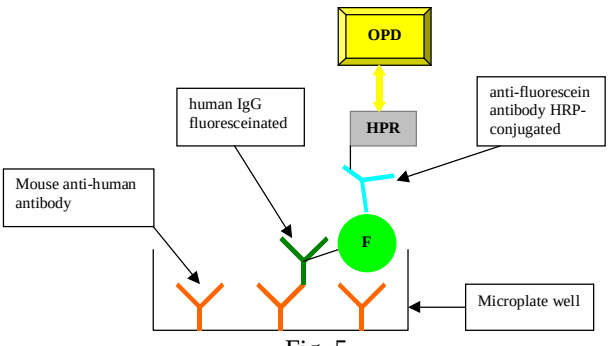

Fig. 5

The colorimetric development signal was automatically read in kinetics and at end-point by an ANTHOS 2001 spectrophotometric microplate reader.
A specific application of the Quanti Kin software which performs an accurate quantitation after test validation was used. This software allows the quantification of any analyte by interpolation of the curves describing the colour produced by a set of calibration wells whose concentration is known. The colour produced by a sample well is compared with the calibration curves and a quantification is given with a five log range.

III. 2D Model for a diffusive process

Our diffusive model was based on the second Fick's law, which describe the diffusive process by using the concentration gradient c.

$$\frac{\partial c}{\partial t} = D * \nabla c$$

Specifically for the 2D case:

$$\frac{\partial c}{\partial t} = D * \left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right)$$

Where x and y are the main directions of the diffusion.
We have make the above formula discrete by using the finite element analysis approach, obtaining the diffusion plane represented in fig 6.

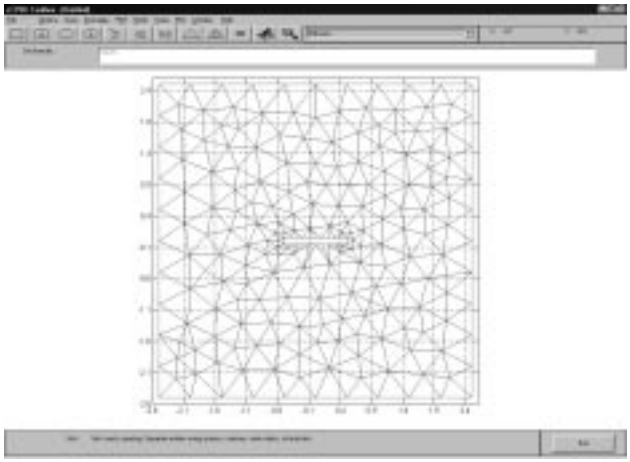

Fig. 6 – Finite Element division of the diffusion plane.

Simulations were performed by the PDE Tool, within MATLAB 5.3 on a Pentium III, 700MHz Pc. Simulation time was in the order of 10 minutes.

IV Preliminary results

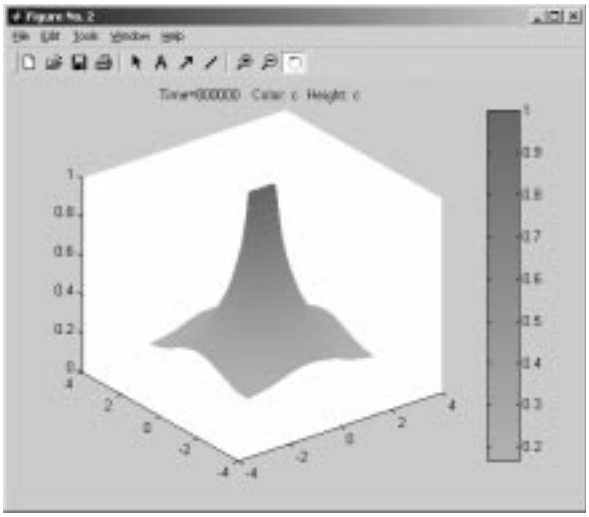

Fig. 7: Diffusion scheme, after 800,000s

We set up the model within Matlab environment and we simulated the diffusion of IgG, taking into account diffusion coefficients present in literature (from 1.11 to 2.19 x 10^{-6} cm^2/s). We calculated the latency time (defined as the time necessary to have 10% of maximum IgG concentration at the boundary of the considered space). This time was found to be in inverse relation to the considered coefficient (from 6300 down to 5000 s). The values obtained by simulation are consistent with experimental results present in the literature [3, 4].
A first set of experiments gave raw data in good agreement with the ones obtained by the model. These preliminary results showed that the halfing time of the concentration within the tube is about three days.

Acknowledgment

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